REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested. Pursuant to 37 CFR § 1.121, attached as an appendix is a version of the amendments with markings to show changes that have been made.

Interactions between bacterial pathogens and their plant hosts generally fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

The hypersensitive response ("HR") is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens. The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp*. Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable. Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia, Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases. In *E. amylovora, P. syringae*, and *P. solanacearum*, hrp genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response.

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin. Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain. However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among gram-negative plant pathogens. Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi*, *Erwinia carotovora*, *Erwinia stewartii*, and *Pseudomonas syringae* pv. *syringae*.

The present invention is a further advance in the effort to identify, clone, and sequence hypersensitive response elicitor proteins or polypeptides from plant pathogens.

Applicants submit that the oath or declaration is not defective. The copies signed by inventors Alan Collmer (inventor 201) and James Alfano (inventor 203) are not altered. The copy signed by inventor Amy Charkowski (inventor 202) was altered by Ms. Charkowski to reflect her correct address at the time the declaration was signed by her. The alteration was initialed by her (see right margin, initials "AOC") contemporaneous with her signing of the declaration. Therefore, these same declarations which were considered by the U.S. Patent and Trademark Office ("PTO") to be effective for the parent application also should be considered effective for the present application. For these reasons, the objection to the declaration should be withdrawn.

Enclosed herewith are 3 sheets of corrected formal drawings to overcome the objections set forth on form PTO 948. The specification has also been amended to reflect changes in the numbering of figures. Therefore, the objection to the drawings should be withdrawn.

The title has been changed, thereby overcoming this objection to the application. This objection, too, should be withdrawn.

The rejection of claims 1-16 and 29-31 under 35 U.S.C. § 112, first paragraph, for lack of enablement is rendered moot with respect to claims 11-16 and 29-31 (cancelled without prejudice) and respectfully traversed with respect to claims 1-10.

Applicants have amended claim 1 to recite the hybridization conditions under which a claimed DNA molecule will hybridize to the complement of SEQ. ID. No. 1. Applicants submit that one of ordinary skill in the art can readily perform hybridization protocols using the recited conditions or comparable conditions modified according to one or more known factors affecting hybridization (i.e., Na^+ concentration, temperature, probe size, formamide concentration, etc.).

The invention of claim 1 recites an "isolated DNA molecule encoding a hypersensitive response eliciting protein or polypeptide." The HR phenomena results from an incompatible interaction between pathogens and their non-host plants. As explained in Gopalan, et. al., "Bacterial Genes Involved in the Elicitation of Hypersensitive Response and Pathogenesis," Plant Disease 80: 604-10 (1996)("Gopalan"), attached hereto as Exhibit 1, this interaction involves a bacterium attempting to infect a host plant, preventing multiplication and spreading of the pathogen, and collapse of plant leaf cells and cell death at the site of infection. This is distinct from a compatible interaction between bacteria and a plant where the bacteria spread in the infected plant, leading to disease symptoms throughout the plant. Id. at 604. Thus, if the protein or polypeptide encoded by the DNA molecule does not elicit a hypersensitive response (i.e., incompatible interaction) when exposed to non-host plants, the encoded protein or polypeptide does not satisfy the limitations of the claimed invention. To determine whether or not a particular protein elicits a hypersensitive response in non-host plants, the protein or polypeptide is applied directly to the plant leaves (see Example 11 and Fig. 5 of the present application).

Contrary to the PTO assertion that the present application does not "teach the specific function of the instant gene", applicants submit that the present application defines a function of the present gene as well as characteristics which are shared among the HrpW protein of the present invention (encoded by claimed DNA molecule of SEQ. ID. No. 1) and other known hypersensitive response elicitors. Beginning at page 27, line 29, it is noted that the HrpW protein is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids. Moreover, in Example 11, it is reported that the HrpW protein is heat-stable, protease sensitive, and capable of eliciting an HR in tobacco leaves.

It is well recognized in the art that hypersensitive response elicitors have a number of common characteristics. These include their being glycine rich, heat stable,

hydrophilic, capable of inducing a hypersensitive response in tobacco after recombinant expression, susceptible to proteolysis, and cysteine lacking. See U. Bonas, "Bacterial Home Goal by Harpins," Trends Microbiol. 2: 1-2 (1994)("Bonas I"), attached hereto at Exhibit 2; U. Bonas, "*hrp* Genes of Phytopathogneic Bacteria," Current Topics in Microbiology and Immunology 192: 79-98 (1994)("Bonas II"), attached hereto as Exhibit 3; and G. Preston, et. al., "The HrpZ Proteins of *Pseudomonas syringae* pvs. *syringae, glycinea,* and *tomato* are Encoded by an Operon Containing *Yersinia ysc* Homologs and Elicit the Hypersensitive Response in Tomato but not Soybean," MPMI 8(5): 717-32 (1995)("Preston"), attached hereto as Exhibit 4.

Moreover, using a DNA molecule of SEQ. ID. No. 1 as a probe for high stringency gel blot hybridization with EcoI-digested DNA from representative necrogenic Gram-negative plant pathogens, the *hrpW* probe hybridized to at least one distinct band for a number of *Pseudomonas syringae* pathovars (e.g., *glycinea*, *papulans*, *pisi*, *phaseolicola*, *tabaci*, and *syringae* strains B728 and 61); *Pseudomonas viridiflava*; *Pseudomonas solanacearum*; and *Xanthomonas campestris* pathovars *amoraciae* and *vesicatoria* (Example 10).

Thus, one of ordinary skill in the art would be fully able to perform a hybridization experiment using the DNA molecule of SEQ. ID. No. 1, and one of ordinary skill in the art would be fully able to prepare the encoded protein and determine whether the encoded protein does, in fact, elicit a hypersensitive response.

For all of the above reasons, applicants submit that the rejection of claims 1-10 under 35 U.S.C. § 112, first paragraph, for lack of enablement should be withdrawn.

The rejection of claims 1-16 and 29-31 under 35 U.S.C. § 112, first paragraph, for lack of written descriptive support is rendered moot with respect to claims 11-16 and 29-31 (cancelled without prejudice) and respectfully traversed with respect to claims 1-10.

Pursuant to the "Guidelines for Examination of Patent Applications Under the 35 U.S.C. § 112, ¶ 1, 'Written Description' Requirement," 66 Fed. Reg. 1099, 1106 (2001), the written description requirement for a genus can be satisfied by "sufficient description of a representative number of species by actual reduction to practice...or by disclosure of relevant identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between structure and function, or by a combination of such identifying characteristics...." Whether a representative number of species is identified depends on whether the disclosed species

represent(s) the entire genus. Id. Applicants submit that these requirements are satisfied by the present application.

In particular, applicants have identified a single representative species which possesses the nucleotide sequence of SEQ. ID. No. 1 and encodes the protein of SEQ. ID. No. 2. The HR elicitor protein encoded by this representative species from *Pseudomonas syringae* pv. *tomato* DC3000 is defined in the present application as possessing the following properties: a molecular mass of about 42.9 kDa (page 9, line 23); an amino acid sequence which is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids (page 27, lines 29-31); heat-stability, protease sensitivity, and capability of eliciting an HR in tobacco leaves (Example 11); and C-terminal homology to Pel domains but lacking Pel activity (Example 12).

As noted above, it is well established that hypersensitive response elicitors as a class possess similar characteristics, including heat stability, protease sensitivity, high glycine content, substantially no cysteine, etc. as described above (see Bonas I, Bonas II, and Preston). The representative species disclosed in the present application shares these characteristics with other members of the larger class of HR elicitors and one of ordinary skill in the art would expect other members of the claimed genus to also possess these characteristics. Moreover, as noted above, using a DNA molecule of SEQ. ID. No. 1 as a probe for high stringency hybridization, the probe hybridized to at least one distinct band for a number of *Pseudomonas syringae* pathovars, *Pseudomonas viridiflava*, *Pseudomonas solanacearum*, and a pair of *Xanthomonas campestris* pathovars (Example 10). Applicants indicated that these hybridization results are significant, because they suggest that *Pseudomonas viridiflava* and *Xanthomonas campestris* produce a protein which is highly similar to HrpW (see page 33, line 29 to page 34, line 2). Thus, applicants demonstrated that the single demonstrated species encodes a HrpW protein which possesses a combination of identifying characteristics that are representative of the protein encoded by DNA molecules of the presently claimed genus. Moreover, the demonstration of hybridization with DNA derived from other pathogens indicates that *hrpW* is conserved at least among various pathogenic *Pseudomonas* species and *Xanthomonas* species. One of ordinary skill in the art clearly would recognize that applicants were in possession of the presently claimed genus.

The PTO has provided no evidentiary basis to believe that the single disclosed species is not representative of the claimed genus and, therefore, the rejection of claims 1-10 for lack of written descriptive support should be withdrawn.

The rejection of claims 1-16 and 29-31 under 35 U.S.C. § 112 (second paragraph) for indefiniteness is rendered moot with respect to claims 11-16 and 29-31 (cancelled without prejudice) and respectfully traversed with respect to claims 1-10.

The rejection of claims 1-10 under 35 U.S.C. § 102(b) as anticipated by Lorang et al., "Characterization of AvrE from *Pseudomonas syringae* pv. tomato: A hrp-linked Avirulence Locus Consisting of at Least Two Transcriptional Units," MPMI 8(1):49-57 (1995) ("Lorang") is respectfully traversed.

Lorang relates to the avirulence protein AvrE from *Pseudomonas syringae* pv. tomato and its encoding gene. Specifically, in Figure 3 of Lorang, a nucleotide sequence is shown which contains two distinct transcriptional units, IV and V. Lorang reports that transcriptional unit IV was necessary for *avrE* activity, while transcriptional unit V was not. Lorang identifies no data which identifies the physiological role of the proteins or polypeptides encoded by transcriptional units IV and V. Lorang at Figure 3 discloses only a partial nucleotide sequence of the DNA molecule of SEQ. ID. No. 1.

Claim 1 presently recites: "An isolated DNA molecule encoding a hypersensitive response eliciting protein or polypeptide, wherein the isolated DNA molecule is selected from the group consisting of (a) a DNA molecule comprising SEQ. ID. No. 1, (b) a DNA molecule encoding a protein comprising SEQ. ID. No. 2, (c) a DNA molecule from a source other than *Pseudomonas syringae* pv. *tomato* which hybridizes to a DNA molecule comprising the complement of SEQ. ID. No. 1 under conditions comprising hybridization at a temperature of about 65°C in a hybridization medium comprising about 1M NaCl, and (d) a DNA molecule complementary to DNA molecules (a), (b), or (c)."

Since Lorang does not teach a DNA molecule comprising SEQ. ID. No. 1, a DNA molecule encoding a protein comprising SEQ. ID. No. 2, a DNA molecule from a source other than *Pseudomonas syringae* pv. *tomato* which hybridizes to a DNA molecule comprising the complement of SEQ. ID. No. 1, or complements thereof, Lorang fails to teach each and every limitation of the claimed invention. Therefore, the rejection of claims 1-10 as anticipated by Lorang should be withdrawn.

The rejection of claims 1-12 and 14-15 under 35 U.S.C. § 102(a) as being anticipated by Tabakaki et al., "Expression of the *Pseudomonas syringae* pv. *phaseolicola hrpZ* Gene in Transgenic Tobacco and *Saccharomyces cerevisiae*," In Developments in Plant Pathology: Pseudomonas Syringae Pathovars and Related Pathogens, Rudolph et al. (eds.),

Kluwer Acad. Publ. (Norwell, MA) pp. 392-396 (1997) ("Tabakaki") is rendered moot with respect to claims 11-12 and 14-15 (now cancelled without prejudice) and respectfully traversed with respect to claims 1-10. The PTO cites Tabakaki for the proposition that the *hrpZ* gene would share at least one nucleotide with SEQ. ID. No. 1 and its encoded protein would share at least one amino acid with the protein of SEQ. ID. No. 2. Applicants submit that the PTO's position is obviated by the above amendments, because Tabakaki does not teach a DNA molecule as recited in claim 1. Therefore, the rejection of claims 1-10 should be withdrawn.

The rejection of claims 1-16 and 29-31 under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 5,850,015 to Bauer et al. ("Bauer '015 patent") is rendered moot with respect to claims 11-16 and 29-31 (now cancelled without prejudice) and respectfully traversed with respect to claims 1-10. The PTO cites to the Bauer '015 patent the proposition that the *hrpN*$_{Ech}$ gene would share at least one nucleotide with SEQ. ID. No. 1 and its encoded protein would share at least one amino acid with the protein of SEQ. ID. No. 2. Applicants submit that the PTO's position is obviated by the above amendments, because the Bauer '015 patent does not teach a DNA molecule as recited in claim 1. Therefore, the rejection of claims 1-10 should be withdrawn.

The rejection of claims 1-16 and 29-31 under 35 U.S.C. § 103(a) for obviousness over Lorang in view of the Bauer '015 patent is rendered moot with respect to claims 11-16 and 29-31 (now cancelled without prejudice) and respectfully traversed with respect to claims 1-10.

Lorang and the Bauer '015 patent are cited substantially as described above.

For the reasons noted above, neither Lorang nor the Bauer '015 patent individually teach or suggest all limitations of the presently recited DNA molecule of claim 1. Even if one of ordinary skill in the art were to combine the teachings thereof, which applicants do not admit is proper, the combination of Lorang and the Bauer '015 patent would still fail to teach or suggest the DNA molecule as recited in claim 1. Specifically, the combination of these references fails to teach or suggest the DNA molecule of SEQ. ID. No. 1, the protein of SEQ. ID. No. 2, a DNA molecule from a source other than *Pseudomonas syringae* pv. *tomato* which hybridizes to the complement of SEQ. ID. No. 1 under the recited conditions, or complements thereof. Thus, the combination of Lorang and the Bauer '015 patent could not have rendered claim 1 obvious, let alone claims 2-10

dependent thereon. For these reasons, the rejection of claims 1-10 for obviousness over Lorang in view of the Bauer '015 patent should be withdrawn.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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3/18/02 Date | Wendy L. Harrold

Appendix
Version With Markings to Show Changes Made

In reference to the amendments made herein to the specification and claims, additions appear as underlined text while deletions appear as strikeout text, as indicated below:

In the Specification:

At page 6, lines 14-20:

Figures 5A-F shows the elicitation in tobacco leaves of active tissue death indicative of the HR by cell-free preparations containing HrpW and the N-terminal fragment. The protein preparations analyzed in Fig. 4 were infiltrated into tobacco leaves, in some cases with 1.0 mM Lanthanum chloride. Leaves were photographed 48-hr later. ~~Panels: A.~~ Figure 5A, *P. syringae* pv. *syringae* 61 HrpZ (0.12 μg/ml); Figure 5B, HrpW; Figure 5C, harpin domain fragment of HrpW (0.22 μg/ml); Figure 5D, HrpZ + lanthanum chloride; Figure 5E, HrpW + lanthanum chloride; Figure 5F, Pel domain fragment of HrpW (1.40 μg/ml).

At page 29, line 13 to page 30, line 8:

PCR subclones of *hrpW* were constructed in pQE30 to permit production of derivatives of HrpW and the two domain fragments carrying N-terminal His_6-tags. These fusion proteins were partially purified by Ni-NTA chromatography and analyzed by SDS-PAGE and by immunoblotting with antibodies raised against *P. syringae* pv. *tomato* DC3000 Hrp-secreted proteins (Fig. 4). Anti-HrpW antibodies did bind to the full-length HrpW and to both fragments, but binding to the hypersensitive response elicitor domain fragment was noticeably weaker. Transformants producing HrpW were highly unstable in their maintenance of the plasmid. Thus, HrpW levels were quite low, and Ni-NTA chromatography yielded a preparation that was only partially enriched in HrpW. Nevertheless, the HrpW preparation elicited a hypersensitive response ("HR")-like necrosis in tobacco leaves, which visibly differed from the necrosis elicited by the *P. syringae* pv. *syringae* 61 HrpZ only in developing ca. 12 hr later (~~Fig. 5~~ Figs. 5A-B). The elicitor activity was heat-stable and protease sensitive, and vector control preparations produced no response. The partially purified hypersensitive response elicitor domain fragment also elicited a necrosis that was slightly delayed, and this response, like that elicited by HrpZ, could be inhibited by 1.0 mM lanthanum chloride, a calcium channel blocker (~~Fig. 5~~ Figs. 5D-E).

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Thus, the necrosis elicited by the HrpW harpin domain is an active plant response. In contrast, purified *E. chrysanthemi* PelE, obtained from *E. coli* JA-221(pPEL748) (Keen, N.T., et al., J. Bacteriol., 168:595-606 (1986), which is hereby incorporated by reference) elicited a black, macerated necrosis that was not inhibited by 1.0 mM lanthanum chloride, 50 μM sodium vanadate, or 100 μM cycloheximide. This is consistent with the expectation that pectic enzymes kill by lysis of turgid protoplasts through weakened cell walls rather than by elicitation of cell death programs. Furthermore, the Pel domain fragment elicited no visible response in the infiltrated tobacco tissue. All three proteins were tested for Pel activity by using the sensitive A_{230} assay for 4,5-unsaturated pectic products (Collmer, A., et al., Meth. Enzymol., 161:329-35 (1988), which is hereby incorporated by reference). No activity was detected despite trying polygalacturonic acid and a 31% methylesterified derivative as substrates, $CaCl_2$ and $MnCl_2$ as cofactors, and several pH levels.

In the Claims:

1. (Amended) An isolated DNA molecule encoding a hypersensitive response eliciting protein or polypeptide, wherein the isolated DNA molecule is selected from the group consisting of (a) a DNA molecule comprising ~~a nucleotide sequence of~~ SEQ. ID. No. 1, (b) a DNA molecule encoding a protein comprising ~~an amino acid of~~ SEQ. ID. No. 2, (c) a DNA molecule from a source other than *Pseudomonas syringae* pv. *tomato* which hybridizes to a DNA molecule comprising ~~a nucleotide sequence~~ the complement of SEQ. ID. No. 1 under ~~stringent~~ conditions comprising hybridization at a temperature of about 65°C in a hybridization medium comprising about 1M NaCl, and (d) a DNA molecule complementary to DNA molecules (a), (b), ~~and~~ or (c).

2. (Amended) An isolated DNA molecule according to claim 1, wherein said DNA molecule is a DNA molecule comprising ~~a nucleotide sequence of~~ SEQ. ID. No. 1.

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3. (Amended) An isolated DNA molecule according to claim 1, wherein said DNA molecule is a DNA molecule encoding a protein comprising ~~an amino acid of~~ SEQ. ID. No. 2.

4. (Amended) An isolated DNA molecule according to claim 1, wherein said DNA molecule is a DNA molecule from a source other than *Pseudomonas syringae* pv. *tomato* which hybridizes to a DNA molecule comprising ~~a nucleotide sequence~~ the complement of SEQ. ID. No. 1 under conditions comprising hybridization at a temperature of about 65°C in a hybridization medium comprising about 1M NaCl.

5. (Amended) An isolated DNA molecule according to claim 1, wherein said DNA molecule is a DNA molecule complementary to DNA molecules (a), (b), ~~and~~ or (c).

6. (Amended) An expression vector ~~transformed with~~ comprising the DNA molecule of claim 1.

7. (Amended) An expression vector according to claim 6, wherein the DNA molecule is in ~~proper~~ sense orientation~~ and correct reading frame~~.

9. (Amended) A host cell according to claim 8, wherein the host cell is ~~selected from the group consisting of~~ a plant cell or a bacterial cell.

10. (Amended) A host cell according to claim 8, wherein the DNA molecule is ~~transformed with~~ comprised within an expression vector.